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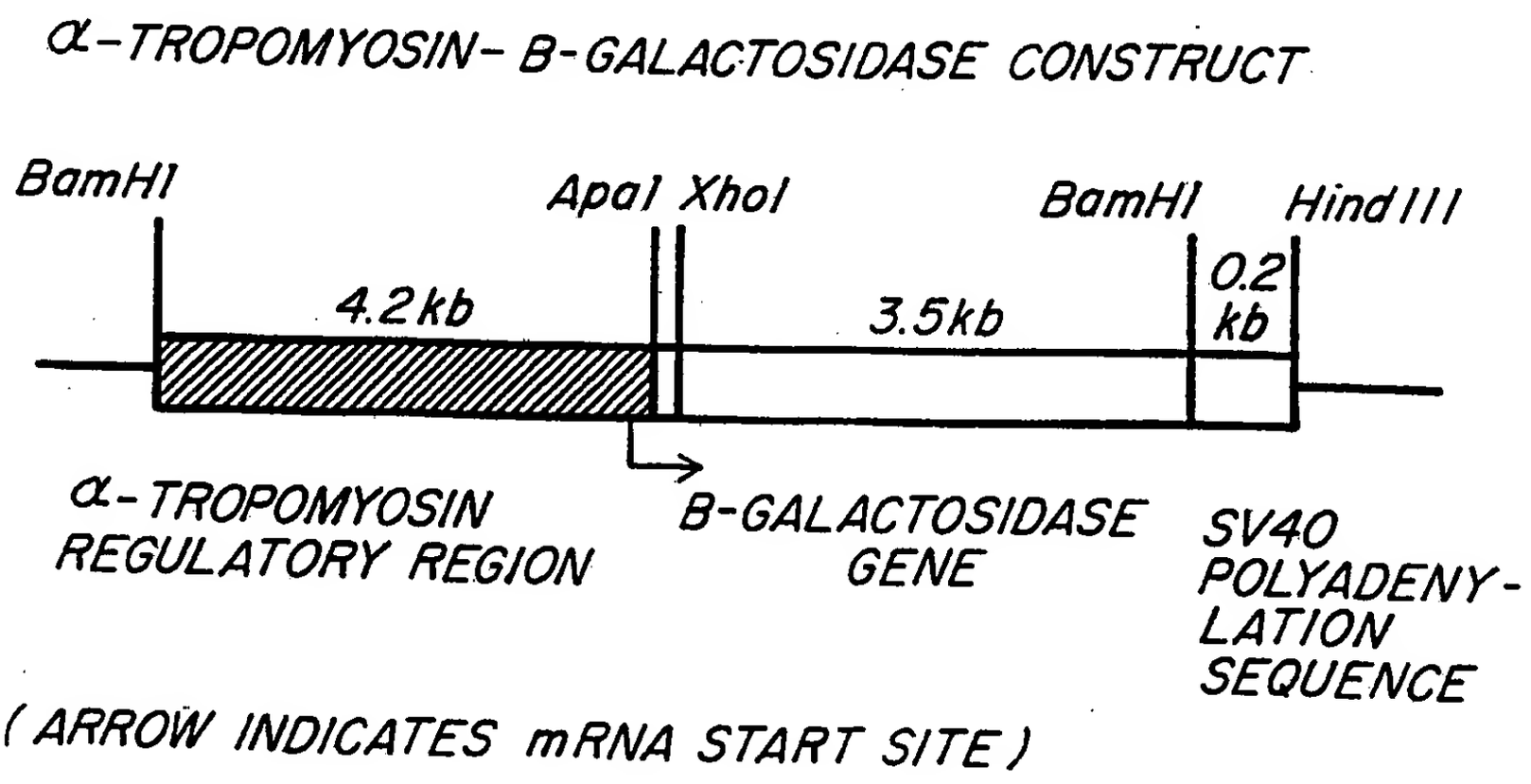

(57) Abstract

An *in vivo* method is provided for testing the ability of a drug to interfere with the onset or maintenance of neoplastic transformation. A drug is administered to a tumor-bearing mammal. The mammal has a transformation-sensitive reporter unit. The reporter unit includes a structural gene, the expression of which is turned on or off depending upon whether cells of the mammal are in a neoplastic state. It then is determined whether the drug has reversed or interfered with the effects of transformation by assaying for the expression of the structural gene. This method permits detection of anti-neoplastic effects by drugs that are noncytotoxic at the doses administered. Transgenic animals and cell cultures also are provided.

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ANTI-NEOPLASTIC IN VIVO DRUG SCREEN

Field of the Invention

This invention relates to a novel, in vivo drug screen for identifying anti-neoplastic agents and to transgenic animals relating thereto.

Background of the Invention

Locating drugs to combat cancer has been a focus of medical research for many years, involving billions of dollars. Typical drug screening assays (and drug approaches to treating cancer) target drugs that are cytotoxic to cancer cells. This strategy generally has severe drawbacks and limitations, including the inability of such assays to identify drugs that are not cytotoxic to cancer cells, yet are effective anticancer agents.

Typical drug screening assays also are in vitro systems (1, 2, 3, 4, 5). One recently developed in vitro system uses a fibroblast cell line including a transformation-sensitive promoter fused to an E. coli reporter gene (6). In an untransformed cell, the reporter gene is expressed; in a transformed cell, the reporter gene is repressed. It is suggested that this assay can be used to identify potential candidates for cancer therapy. That is, if the potential candidate is applied to a transformed cell and reverses the repression characteristic of the transformed state, then the drug possibly may work in vivo for some unidentified cancer state. This system, though interesting, has nearly all of the drawbacks of any other in vitro system. It provides no information about what happens in vivo, including, for example, no information about drug availability, dosing regimen to identify and maintain active drug concentration, tissue specificity, in vivo drug modification, and age related issues.

Summary of the Invention

According to one aspect of the invention, an in vivo method is provided for testing the ability of a drug to interfere with the onset or maintenance of neoplastic transformation. A drug is administered to a tumor-bearing animal. The animal has a transformation-sensitive reporter unit. The reporter unit includes a structural gene, the expression of which is turned on or off depending upon whether cells of the animal are in a neoplastic state. It then is determined whether the drug has reversed or interfered with the effects of transformation by assaying for the expression of the structural gene. This method permits detection of anti-neoplastic effects by drugs that are noncytotoxic at the doses administered.

Preferably, the drug is administered to a novel, transgenic, nonhuman animal that has a transformation sensitive reporter unit, at least a portion of which is introduced stably into the animal, or an ancestor of the animal, preferably (but not necessarily) at an embryonic stage. The transformation sensitive reporter unit includes a regulatory region that is either repressed upon neoplastic transformation of cells of the animal or activated upon such neoplastic transformation. Preferably, the reporter unit includes an exogenous reporter structural gene. Most preferably, the foregoing transgenic animals are selected for or provided with a predisposition to develop neoplasia.

According to another aspect of the invention, methods for forming novel cell cultures are provided. A transgenic nonhuman animal is created, whose germ cells and/or somatic cells contain a recombinant, transformation sensitive reporter unit introduced into the animal, or an ancestor of the animal, preferably (but not necessarily) at an embryonic stage. Then, one or more of the somatic cells of the transgenic nonhuman animal are cultured.

These and other aspects of the invention, as well as the advantages of the invention, will be more apparent from the following detailed description of the invention.

Brief Description of the Drawings

Fig. 1 depicts a preferred, transformation-sensitive, reporter unit.

Fig. 2 depicts a preferred construct for creating a transgenic animal with a predisposition to develop neoplasia.

Detailed Description

The invention involves a novel, living reporter system that is a model for the investigation of neoplastic transformation and the reversal thereof. It is based upon the provision of an animal that has a transformation sensitive reporter unit which produces a different signal depending upon whether or not cells of the animal are in a neoplastic state. Preferably, the animal has the genetic trait of being predisposed to develop neoplasia. A secondary oncogenic agent or event may be presented to the animal to accelerate the induction of cellular transformation. Using the above-described animal, the neoplastic condition can be monitored by assaying for the presence or absence of the signal. This provides an ideal model for drug screening, in that drugs can be administered to an animal, for example, that has already developed a tumor, and then the ability of the drug to act as an anti-neoplastic agent can be measured by detecting the ability of the drug to affect the signal from the reporter system. The reporter system can be chosen so that the efficacy of the drug can be easily determined, for example, by the presence or absence of a color. A color change would occur considerably sooner than a reduction in tumor size.

This invention thus provides a mammalian model of the development of neoplastic transformation that is readily assayable. The term neoplastic transformation is intended to include one or more events in a cell that initiate as well as contribute to the development of a tumor or cancerous state which may include the loss of cell growth control and redirection of the normal physiological processes of the

cell. The terms hyperplasia, metaplasia, and dysplasia are commonly used to describe stages in the development of neoplastic transformation. By "development" it is meant onset, maintenance or progression.

While previous antineoplastic drug screens have focused on identifying agents that kill rapidly growing cells, this invention permits the identification in an animal or a cell derived from an animal of drugs with different properties. It can identify a drug or a dose of a drug that reverses the transformed phenotype, rather than only those drugs or doses of drugs that promote cell death. Therefore, novel classes of anti-cancer drugs with fewer detrimental side-effects may be identified.

This invention further provides access to obtaining information about drug availability. It can be determined if the drug reaches the tumor, the extent to which the drug penetrates the tumor, if the drug has different effects on different size tumors, if the drug has different effects on tumors at different stages of progression, and if the drug's effects depend upon the state of vascularization of the tumor. For example, differential staining patterns within a tumor (e.g., whether the borders or centers are stained) resulting from a reporter system that produces a color change if the drug reverses transformation, can provide useful information on drug delivery. Information about the tissue-specificity of a drug also can be obtained. For example, differential staining can indicate if a drug acts differently on tumors in different tissues.

The proper dosing of an anti-cancer drug also can be determined. In the preferred embodiment, the assay identifies a putative anti-cancer drug if the tumor changes color, even if the tumor might not have undergone a reduction in size. Once such a candidate drug is identified, the doses and/or timing of drug administration can be manipulated to achieve a desired result.

In addition, the invention allows an assessment of

whether external conditions interfere with the efficacy of the drug. Unlike in vitro screens, this invention permits analysis of conditions specific to live animals, e.g., the animal's age, the animal's health, the degree of vascularization near the tumor, synergistic effects with other drugs or nutrients ingested by the animal, and the state of the animal's immune system. Moreover, the invention can permit a determination of whether the efficacy of the drug depends upon in vivo modification of the drug.

The invention thus also provides novel transgenic animals. By animals it is meant vertebrate animals including birds, fish, amphibians and mammals. Preferred animals are mammals and particulary preferred mammals are rabbits, horses, cows, goats, sheep, dogs, cats, rodents and monkeys.

As discussed above, the animal preferably has a predisposition to develop neoplasia. By predisposition to develop neoplasia, it is meant that the animal is in a condition where tumor development is more likely to occur, as compared to an animal of the same type which is not predisposed to develop neoplasia. The predisposed condition is the result of a primary agent or event.

Transgenic animal lines exist which have such a predisposition to develop tumors. The term transgenic animal is meant to include an animal that gains new genetic information from the introduction of foreign DNA into its cells. The animal may include the transgene in all of its cells including germ line cells, or in only some of its cells such as in selected somatic cells. An example of an existing transgenic animal line with a predisposition to develop tumors is U.S. Patent 4,736,866, issued April 12, 1988 to Leder, the entire contents of which are incorporated herein by reference.

Animal lines with a predisposition to develop tumors can be created in a variety of ways, including:

(i) germline introduction of various genes encoding primary cancer causing events under the transcriptional

control of homologous or heterologous promoters, including genes such as activated oncogenes, viral genes (e.g. SV40 T-antigen, polyoma T-antigen, Tat), proto-oncogenes and inactivated tumor suppressors (e.g., NF1 mutant, p53 mutant); (ii) germline ablation or inactivation (interruption or deletion) by homologous recombination or antisense expression of tumor suppressor genes (e.g., RB, DCC, NF1); (iii) treatment of animals, neonates, or animals in utero with carcinogens and/or mutagens, e.g., NMU; (iv) activation of a proto-oncogene by the integration of a viral genome (of a retrovirus) adjacent to the proto-oncogene; (v) treatment of animals with sex hormones (e.g., estrogens or testosterones); and (vi) breeding.

A secondary event or agent is generally required to activate a predisposed animal to actually develop tumors. Examples of such agents or events are:

(i) introduction of a second activating oncogene giving rise to a phenomenon called oncogene synergy or cooperation; (ii) introduction of an activating oncogene into an animal with an existing preneoplastic state resulting from an inactivated tumor suppressor gene function; (iii) treatment with carcinogens, e.g., NMU to activate an oncogene; (iv) tumor promoting agents like phorbol esters (e.g., phorbol 12-myristate 13-acetate); (v) tissue injury; (vi) hormonal treatment (or sexual maturity), e.g., for breast and prostate carcinomas; (vii) exposure to radiation; and (viii) spontaneous somatic mutations resulting in tumor formation.

An oncogene is a gene whose product when expressed within a eukaryotic cell has the ability to transform that cell. Examples of classes of oncogenes are growth factors/mitogens, receptors, cytoplasmic or membrane associated proteins, nuclear proteins/transcription factors, and certain unclassified genes. Growth factors/mitogens include hst, int2 and sis. Other potential growth factors/mitogens are FGF-5, CSF-1, GM-CSF, PDGF, TGFα and EGF. Receptors include protein tyrosine kinases, e.g., erbB, fms, kit, met,

ret, ros, sea, trk and neu; and non-protein kinases, e.g., mas. Cytoplasmic and membrane associated proteins include tyrosine kinases, e.g., abl, fes, fgr, fps, lck, src and yes; G-proteins, e.g., H-ras, K-ras, N-ras, gip and gsp; and protein serine threonine kinases, e.g., raf, pim-1, mos and PKC. Nuclear proteins/transcription factors include erbA, ets, fos, fos-B, fra-1, jun, junB, junD, myb, myc, L-myc, N-myc, rel, ski, vav, evi-1, gli-1, maf, pbx and Hox2.4. Unclassified oncogenes include dbl and bcl-2.

Examples of sources of transforming viral genes include oncogenic RNA and DNA viruses. Oncogenic RNA viruses include Rous Sarcoma Virus (Chicken), Y73 Sarcoma Virus (Chicken), Fujinami (Feline) Sarcoma Virus (Cat), Abelson Murine Leukemia Virus (Mouse), Rochester-2 Sarcoma Virus (Chicken), Gardner-Rasheed Feline Sarcoma Virus (Cat), Avian Erythroblastosis Virus (Chicken), McDonough Feline Sarcoma Virus (Cat), Moloney Murine Sarcoma Virus (Mouse), 3611 Murine Sarcoma Virus (Mouse), Harvey Murine Sarcoma Virus (Rat and Mouse), Kirsten Murine Sarcoma Virus (Rat), Avian MC29 Myelocytomatosis Virus (Chicken), Avian Myeloblastosis Virus (Chicken), FBJ Osteosarcoma Virus (Mouse), Avian SKV700 Virus (Chicken), Reticuloendotheliosis Virus (Turkey), Simian Sarcoma Virus (Woolly Monkey), Chicken AEV (Chicken), Rasheed Sarcoma Virus (Rat), Carcinoma Virus MH2 (Chicken), Avian myeloblastosis/erythroblastosis Virus E26 (Chicken), and Erythroblastosis virus (Chicken). Oncogenic DNA viruses include SV40, adeno, polyoma, herpes virus saimiri, Epstein-Barr, hepatitis B, human papillomavirus and herpes simplex virus.

A proto-oncogene is a normal cellular gene that can be activated to a dominant-acting oncogene (7). Proto-oncogenes can be activated by one of the following mechanisms:

1. Point mutation sustained by naturally occurring events.

2. Point mutation sustained by mutagen/carcinogen treatment of cells or animals (8), irradiation, or in vitro site-directed mutagenesis.

3. Intragenic deletion (9).

4. Over-expression, e.g., mutation in the transcriptional regulatory regions of the gene or insertion of a retroviral genome into the proto-oncogene locus. An example is the over-expression of the secreted oncogene products sis or hst (10).

5. Gene rearrangement (11).

Examples of proto-oncogenes which are associated with tumors in specific human tissues are listed in Table 1.

Table 1

Proto-Oncogene	Neoplasm
ABL	Chronic myelogenous leukemia
ERBB-1	Squamous cell carcinoma; astrocytoma
ERBB-2 (NEU)	Adenocarcinoma of breast, ovary and stomach
GIP	Carcinoma of ovary and adrenal gland
GSP	Adenoma of pituitary gland; carcinoma of thyroid
MYC	Burkitt's lymphoma Carcinoma of lung, breast and cervix
L-MYC	Carcinoma of lung
N-MYC	Neuroblastoma; small cell carcinoma of lung
H-RAS	Carcinoma of colon, lung and pancreas; melanoma
K-RAS	Acute myelogenous and lymphoblastic leukemia; carcinoma of thyroid; melanoma
N-RAS	Carcinoma of genitourinary tract and thyroid; melanoma
RET	Carcinoma of thyroid
ROS	Astrocytoma
K-SAM	Carcinoma of stomach

SIS	Astrocytoma
SRC	Carcinoma of colon
TRK	Carcinoma of thyroid

Some proto-oncogenes code for receptors involved in the development of particular cell types. Examples of receptors that when mutated result in oncogenesis include the erthropoietin receptor (12), the α1ß adreneric receptor (13) and the EGF receptor (14).

Inactivation of a tumor suppressor gene can result in tumor formation. Inactivation of tumor suppressor genes can be accomplished through the following methods:

a. Antisense RNA expression (This approach has been successfully used to block specific oncogene expression (15, 16, 17).

b. Gene knockout by homologous recombination (18, 19, 20).

c. Inactivating mutations (point mutations, deletions) introduced in vitro or by carcinogen treatment, e.g., p53 genes (21, 22).

Examples of altered tumor suppressor gene function which are associated with tumors in specific human tissues are listed in Table 2 (7).

Table 2

Tumor Suppressor Gene	Neoplasm
RB1	Retinoblastoma; osteosarcoma; carcinoma of breast, bladder and lung
P53	Astrocytoma; carcinoma of breast, colon and lung; osteosarcoma
WT1	Wilms' tumor
DCC	Carcinoma of colon
NF1	Neurofobromatosis type 1

FAP	Carcinoma of colon
MEN-1	Tumors of parathyroid, pancreas, pituitary and adrenal cortex

A carcinogen is an agent which has the capacity to cause changes directly or indirectly in cells that result in cellular transformation. Examples of carcinogens include N-nitroso-N^1-methyl urea (NMU), 7, 12-dimethylbenz[a]anthracene (DMBA), tetranitromethane (TNM), dibenz[c,h]acridine (DBACR), 1'-hydroxy-2',3'-dehydroestragole (HODE), N-hydroxy-2-acetylaminofluoride (HOAAF), vinyl carbamate (VC), methyl (methoxymethyl) nitrosoamine (DMN), furfural and 3-methyl-cholanthrene (MCA). The following table illustrates the activation of an oncogene, ras, in carcinogen-induced animal tumors, and the tissue specificity for the tumor that is formed (8).

Table 3

Species	Carcinogen	Tumor	Oncogene	Incidence
rat	NMU	Mammary Carcinoma	H-ras-1	86%
	DMBA	Mammary Carcinoma	H-ras-1	23%
	DMN	Kidney mesenchymal	K-ras-2	40%
	TNM	Lung Carcinoma	K-ras-2	74%
mouse	DMBA	Skin Carcinoma	H-ras-1	90%
	DBACR	Skin Carcinoma	H-ras-1	80%
	DMBA	Mammary Carcinoma	H-ras-1	100%
	X-rays	Lymphoma	N-ras, K-ras-2	57%
	NMU	Lymphoma	N-ras, K-ras-2	85%
	MCA	Thymic Lymphoma	K-ras-2	83%
	MCA	Fibrosarcoma	K-ras-2	50%
	HOAAF	Hepatocellular Carcinoma	H-ras-1	100%
	VC	Hepatocellular Carcinoma	H-ras-1	100%
	HODE	Hepatocellular Carcinoma	H-ras-1	100%

Furfural	Hepatocellular Carcinoma	H-ras-1	85%
TNM	Lung Carcinoma	K-ras-2	100%

For example, one of the following combinations of primary and secondary agents or events can be used to improve the efficiency of tumor formation in an animal.
(i) oncogene synergy in spontaneous or accelerated transgenic tumor formation (23):

Table 4

Transgene	Synergistic Gene	Tumor Type
V-abl	myc	Plasmacytoma
bcl-2	myc	Plasmacytoma
myc	N-ras, K-ras	Pre-B lymphoma
SV40 Tag	H-ras	Liver carcinoma
gp55 gene	p53, spi-1	Erythroleukemia
	Retroviral delivery	
myc	v-H-ras, v-raf, N-ras	Pre-B lymphoma
	Transgenic cross	
myc	v-H-ras	Mammary carcinoma
myc	v-abl	Plasmacytoma
myc	v-abl	Pre-B lymphoma
myc	pim-1	Pre-B lymphoma
myc	N-ras	Pre-B lymphoma
myc	bcl-2	Lymphoid progenitor
SV40 Tag	myc	Liver carcinoma
SV40 Tag	H-ras	Gross liver hyperplasia
myc	H-ras	Liver carcinoma

(ii) carcinogen and sex hormone (24):

Table 5

Carcinogen (Primary)	Promotion (Secondary)	Tumor Type
NMU	Testosterone	Prostate carcinomas
NMU	Estrogen	Mammary carcinomas

(iii) tumor suppressors and oncogenes/viruses (25, 26):

Table 6

Tumor Suppressor (Primary)	Oncogene/Virus (Secondary)	Tumor Type
p53	ras	cell culture
p53	friend virus	erthroleukemias

(iv) oncogene and treatment (27, 28, 29):

Table 7

Oncogene (Primary)	Treatment (Secondary)	Tumor
H-ras, TGFα	abrasion or wounding	papillomas
H-ras	Phorbol esters (PMA)	palillomas

The primary and secondary agents and events which are involved in tumor formation can be introduced at various stages during animal development. As will be recognized by those of ordinary skill in the art, the choice of which stage depends at least upon the particular agent or event used and whether it is for a primary or secondary effect.

The animal must have a transformation sensitive reporter unit. A "transformation sensitive reporter unit" requires

sequence of DNA that encodes for a product that can be monitored and also a sequence of DNA through which expression of the product may be regulated in response to cellular transformation. "Regulation of expression" means regulation at the transcriptional or translational level.

Expression of the product of the reporter unit can be either repressed or activated in response to the transformation event. Repression refers to the partial or complete inhibition of expression. Activation refers to the turning on or enhancement of expression. It is important that activation or repression be readily monitored. Therefore, in preferred embodiments the reporter unit is either substantially completely repressed or substantially completely activated by transformation. Monitoring expression of the product can be accomplished by histochemical, immunohistochemical, enzymatic assays or in situ hybridization methods in whole animals, tissue sections, cell homogenates or single cells.

In the case where the expression of the reporter unit is at the transcriptional level, the regulatory region of the transformation sensitive reporter unit is a cis-acting DNA sequence through which transcription of a gene is controlled. A function of this sequence is to be recognized by regulatory proteins. A transcriptional regulatory region includes promoters and enhancers. A promoter is a DNA sequence which directs the start of RNA synthesis. It is a region of DNA that is involved in binding RNA polymerase to initiate transcription. A promoter may be the target of one or many regulatory proteins. Examples of regulatory proteins are repressors and activators. An enhancer is a DNA sequence that in combination with a given promoter in the presence of the appropriate protein(s) directs the increased utilization of that promoter in specific tissue(s). Enhancers can act over considerable distances, at least up to several thousand base pairs, from the start point of transcription. Enhancers can function in either orientation relative to the promoter.

They are position-independent in that they can function upstream or downstream relative to the promoter. A particular enhancer may be a target for tissue specific or temporal regulation.

Examples of reporter units having regulatory regions that are sensitive to transformation by various oncogenes/viruses are shown in the following table.

Table 8

Gene	Oncogene/Virus
α-Actin	H-ras Adeno v-K-ras SV40 E1A
Myosin Heavy Chain	E1A
Myosin Light Chain	v-K-ras
α-Tropomyosin	v-K-ras v-H-ras v-mos v-fms v-fes v-src SV40 RSV
MyoD1	H-ras
Myogenin	TGFB v-fos
Collagen alpha2 (I)	v-mos v-frc v-ras
H-2K (class 1MHC)	E1A Adeno

Neu	E1A
Phosphoenol pyruvate carboxykinase	E1A
Polyoma	E1A

Examples of reporter units having regulatory regions that are activated upon transformation include a large number of genes such as glucose transporter type I (GLUTI) (30) and multidrug resistance gene (MDRI) (31), as well as other as yet uncharacterized genes (32).

In addition to a regulatory region, the transformation sensitive reporter unit contains a structural gene, a sequence of DNA that encodes for a product. The structural gene can code for any RNA or polypeptide product. The product may be a full length gene product, or it may be a subfragment thereof, or it may be part of a fusion product, provided that it is assayable. In the case of interrupted eukaryotic genes, a structural gene is meant to include sequences which include exons and introns, as well as those that include exons and some introns, or only exons.

A factor to be considered in the choice of a structural gene to be used as the reporter for this invention is ease of assayability. Preferred reporters include exogenous structural genes so as to avoid any interference in the assays with endogenous background levels of the reporter gene product.

In the case where the reporter gene is a coding sequence for a protein, whether an exogenous protein or a protein the expression of which is modulated directly by the transformation state of a cell, an antibody specific for that protein can be used to detect expression of the protein. A wide range of antibodies for cellular proteins is available from commercial suppliers such as Sigma Chemical, Boehringer Mannheim, Dako Corp.

Examples of reporter genes are Escherichia coli lac Z

(codes for ß-galactosidase), Escherichia coli CAT (codes for chloramphenicol acetyltransferase), Firefly luciferase (codes for luciferase), and the proteins from the genes listed in Table 8. These reporters can be assayed histochemically, enzymatically (34), immunohistochemically (35), or by in situ hybridization (36). Preferably, ß-galactosidase is assayed histochemically, for example, with X-gal as a color indicator. ß-galactosidase reacts with X-gal to produce a blue color which is easily visualized (33). Luciferase preferably is assayed by chemiluminescence (37). The proteins from the genes listed in Table 8 are assayed by antibody reaction. The most preferred reporter is the Escherichia coli lacZ gene.

The transformation sensitive reporter unit preferably involves a transgene and may be introduced into the animal cell as an intact unit. For example, an exogenous structural gene may be recombined with a regulatory region in vitro to form a transformation sensitive reporter unit. The regulatory region may be derived from a regulatory region that is normally endogenous to the target animal, or from a regulatory region that is exogenous to the target animal. The term endogenous regulatory region is a regulatory region that is normally found in the genome of the non-transgenic target animal. As a result of the in vitro recombination event, the regulatory region is operatively coupled to the structural gene. The term operatively coupled is meant to include the situation where expression of the structural gene is under the control of the regulatory region. Such control includes control by either the promoter or enhancer of the regulatory region, or by both.

Alternatively, the transformation sensitive reporter unit may be formed in vivo as a result of recombination between a part of the unit that is introduced into the animal cell and a part of the unit that is endogenous to the cell. For example, an exogenous reporter structural gene may be introduced into the cell and undergo recombination with the

animal's genome, resulting in insertion of the exogenous structural gene so that it is regulated by an endogenous regulatory region. The term exogenous reporter structural gene is meant to include a gene that is not normally found in the genome of the non-transgenic animal. Preferably, insertion of the exogenous structural gene is recombined into a nonessential region of the genome so that the cell is not killed. Insertion may occur upstream or downstream of the transformation sensitive regulatory region, provided that the regulatory region is operatively coupled to the structural gene.

Insertion of either an intact transformation sensitive reporter unit, or insertion of part of a transformation sensitive reporter unit should be in a manner so as to result in stable introduction into the animal cell. By stable introduction it is meant that the transformation sensitive reporter unit is introduced into a recipient cell and becomes integrated into a resident chromosome or is maintained in successive generations as an autonomously replicating unit. An example of an autonomously replicating unit is Bovine Papilloma Virus Vector (38).

A potential anti-cancer drug is screened using an animal with the following characteristics: (1) all or some of its cells express a transformation sensitive reporter unit as described above and (2) some of the same cells have undergone transformation as described above. In the preferred embodiment, the regulatory region of the transformation sensitive reporter is repressed as a result of transformation and the reporter gene is substantially turned off so as to eliminate detectable product of the reporter gene. An anti-cancer drug of the desired class will reverse the transformed state, resulting in restoration of reporter gene expression. Alternatively, the regulatory region is activated as a result of transformation and the reporter gene is turned on. In such a situation, an anti-cancer drug will result in reversal of reporter gene expression. In either

embodiment, the ability of the drug to reverse expression of the reporter gene, as compared to its expression under transforming conditions, identifies the drug as an anti-cancer drug.

The following non-limiting examples further illustrate the present invention.

EXAMPLES

Sources of Materials

Restriction endonucleases are obtained from conventional commercial sources such as New England Biolabs (Beverly, MA.), Promega Biological Research Products (Madison, WI.), and Stratagene (LaJolla CA.), etc. Radioactive materials are obtained from conventional commercial sources such as Dupont/NEN or Amersham. Custom-designed oligonucleotides for site-directed mutagenesis are available from any of several commercial providers of such materials such as Bio-Synthesis Inc., Lewisville, TX. Kits for carrying out site-directed mutagenesis are available from commercial suppliers such as Promega Biological Research Products, Stratagene, etc. Libraries of DNA are available from commercial providers such as Stratagene, La Jolla, CA., or Clontech, Palo Alto, CA. Rat 2 and N1H 3T3 cells are available from ATCC (#CRL1764 and #CRL1658 respectively). Standard cell culture media appropriate to the cell line are obtained from conventional commercial sources such as Sigma, Gibco/BRL Murine stem cells, strain D3, were obtained from Dr. Rolf Kemler (39). Materials for DNA transfection (40) and the drug G418 for selection of stable transformants are available from Gibco/BRL.

Preparation of Promoter-Reporter Constructs

As described above, various transformation sensitive promoters can be fused to a desired reporter gene using standard molecular biological manipulations (41). The ability of the promoter(s) to direct the expression of the

reporter genes in these "mixed and matched" constructs are evaluated in tissue culture cells (e.g. NIH3T3, Rat2 or other cell lines) in transient and stable transfection experiments. The ability of the promoter-reporter constructs to respond to cellular transformation in tissue culture cell lines also is evaluated. The constructs are then introduced into animals transgenically to determine if the transcriptional characteristics are retained in vivo in the whole animal. The constructs also are evaluated for their ability to direct a predictable/reproducible pattern of tissue expression.

Preparation of Constructs for Transfections and Microinjections

Methods for purification of DNA for microinjection are well known to those or ordinary skill in the art (41, 42, 43, 44).

Construction of Transgenic Animals

A variety of methods are available for the production of transgenic animals associated with this invention. DNA can be injected into the pronucleus of the fertilized egg before fusion of the male and female pronuclei, or injected into the nucleus of an embryonic cell following the initiation of cell division: the nucleus of a two-cell embryo for example. Embryos can be infected with viruses, especially retroviruses, modified to bear transgenes. Pluripotent stem cells derived from the inner cell mass of the embryo and stabilized in culture can be manipulated in culture to incorporate transgenes. A transgenic animal can be produced from such cells through implantation into a blastocyst that is implanted into a foster mother and allowed to come to term.

Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, MA), Taconic (Germantown, NY), Harlan Sprague Dawley (Indianapolis, IN), etc. Swiss Webster female

mice are preferred for embryo retrieval and transfer. $B6D2F_1$ males can be used for mating and vasectomized Swiss Webster studs can be used to stimulate pseudopregnancy. Vasectomized mice and rats can be obtained from the supplier.

Microinjection Procedures

The procedures for manipulation of the rodent embryo and for microinjection of DNA into the pronucleus of the zygote are well known to those of ordinary skill in the art (42). In addition, microinjection of DNA molecules into the nucleus of a two-cell embryo can also be used to produce transgenic animals (45).

Microinjection procedures for fish and amphibion eggs are detailed in (46). Procedures for producing transgenic birds are detailed in (46).

Other DNA Introduction Procedures

Procedures for introduction of DNA into tissues of animals are described in (48).

Transgenic Mice

Female mice six weeks of age are induced to superovulate with a 5 IU injection (0.1 cc, ip) of pregnant mare serum gonadotropin (PMSG; Sigma) followed 48 hours later by a 5 IU injection (0.1 cc, ip) of human chorionic gonadotropin (hCG; Sigma). Females are placed with males immediately after hCG injection. Twenty-one hours after hCG, the mated females are sacrificed by CO_2 asphyxiation or cervical dislocation and embryos are recovered from excised oviducts and placed in Dulbecco's phosphate buffered saline (DPSS) with 0.5% bovine serum albumin (BSA; Sigma). Surrounding cumulus cells are removed with hyaluronidase (1 mg/ml). Pronuclear embryos are then washed and placed in Earle's balanced salt solution containing 0.5% BSA (EBSS) in a 37.5YC incubator with a humidified atmosphere at 5% CO_2, 95% air until the time of injection.

Randomly cycling adult female mice are paired with vasectomized males. Swiss Webster or other comparable strains can be used for this purpose. Recipient females are mated at the same time as donor females. At the time of embryo transfer, the recipient females are anesthetized with an intraperitoneal injection of 0.015 ml of 2.5% avertin per gram of body weight. The oviducts are exposed by a single midline dorsal incision. An incision is then made through the body wall directly over the oviduct. The ovarian bursa is then torn with watchmakers forceps. Embryos to be transferred are placed in DPBS and in the tip of a transfer pipet (about 10-12 embryos). The pipet tip is inserted into the infundibulum and the embryos transferred. After the transfer, the incision is closed by two sutures.

Transgenic Rats

The procedure for generating transgenic rats is similar to that of mice (49). Thirty day-old female rats are given a subcutaneous injection of 20 IU of PMSG (0.1 cc) and 48 hours later each female placed with a proven male. At the same time, 40-80 day old females are placed in cages with vasectomized males. These will provide the foster mothers for embryo transfer. The next morning females are checked for vaginal plugs. Females who have mated with vasectomized males are held aside until the time of transfer. Donor females that have mated are sacrificed (CO_2 asphyxiation) and their oviducts removed, placed in DPSS with 0.5% BSA and the embryos collected. Cumulus cells surrounding the embryos are removed with hyaluronidase (1 mg/ml). The embryos are then washed and placed in EBSS (Earle's balanced salt solution) containing 0.5% BSA in a 37.5YC incubator until the time of microinjection.

Once the embryos are injected, the live embryos are moved to DPBS for transfer into foster mothers. The foster mothers are anesthetized with ketamine (40 mg/kg, ip) and xylazine (5 mg/kg, ip). A dorsal midline incision is made through the

skin and the ovary and oviduct are exposed by an incision through the muscle layer directly over the ovary. The ovarian bursa is torn, the embryos are picked up into the transfer pipet, and the tip of the transfer pipet is inserted into the infundibulum. Approximately 10-12 embryos are transferred into each rat oviduct through the infundibulum. The incision is then closed with sutures, and the foster mothers are housed singly.

Embryonic Stem (ES) Cell Methods

Introduction of DNA into ES cells:

Methods for the culturing of ES cells and the subsequent production of transgenic animals, the introduction of DNA into ES cells by a variety of methods such as electroporation, calcium phosphate/DNA precipitation, and direct injection are well known to those of ordinary skill in the art (50). Selection of the desired clone of transgene-containing ES cells is accomplished through one of several means. Although embryonic stem cells are currently available for mice only, it is expected that similar methods and procedures as described and cited here will be effective for embryonic stem cells from different species as they become available.

In cases involving random gene integration, a transgene clone is co-transfected with a gene encoding neomycin resistance. Alternatively, the gene encoding neomycin resistance is physically linked to the transgene. Transfection is carried out by any one of several methods well known to those of ordinary skill in the art (50, 51). Calcium phosphate/DNA precipitation, direct injection, and electroporation are the preferred methods. Following DNA introduction, cells are fed with selection medium containing 10% fetal bovine serum in DMEM supplemented with G418 (between 200 and 500μ g/ml biological weight). Colonies of cells resistant to G418 are isolated using cloning rings and expanded. DNA is extracted from drug resistant clones

and Southern blotting experiments using a transgene-specific DNA probe are used to identify those clones carrying the transgene sequences. In some experiments, PCR methods are used to identify the clones of interest.

DNA molecules introduced into ES cells can also be integrated into the chromosome through the process of homologous recombination (52). Direct injection results in a high efficiency of integration. Desired clones are identified through PCR of DNA prepared from pools of injected ES cells. Positive cells within the pools are identified by PCR subsequent to cell cloning. DNA introduction by electroporation is less efficient and requires a selection step. Methods for positive selection of the recombination event (i.e., neo resistance) and dual positive-negative selection (i.e., neo resistance and gancyclovir resistance) and the subsequent identification of the desired clones by PCR have been described by (52, 53), the teachings of which are incorporated herein.

Embryo Recovery and ES Cell Injection:

Naturally cycling or superovulated female mice mated with males are used to harvest embryos for the implantation of ES cells. It is desirable to use the C57B strain for this purpose when using mice. Embryos of the appropriate age are recovered approximately 3.5 days after successful mating. Mated females are sacrificed by CO_2 asphyxiation or cervical dislocation and embryos are flushed from excised uterine horns and placed in Dulbecco's modified essential medium plus 10% calf serum for injection with ES cells. Approximately 10-20 ES cells are injected into blastocysts using a glass microneedle with an internal diameter of approximately 20 μm.

Transfer of Embryos to Receptive Females:

Randomly cycling adult female mice are paired with vasectomized males. Mouse strains such as Swiss Webster, ICR

or others can be used for this purpose. Recipient females are mated such that they will be at 2.5 to 3.5 days post-mating when required for implantation with blastocysts containing ES cells. At the time of embryo transfer, the recipient females are anesthetized with an intraperitoneal injection of 0.015 ml of 2.5% avertin per gram of body weight. The ovaries are exposed by making an incision in the body wall directly over the oviduct and the ovary and uterus are externalized. A hole is made in the uterine horn with a 25 gauge needle through which the blastocysts are transferred. After the transfer, the ovary and uterus are pushed back into the body and the incision is closed by two sutures. This procedure is repeated on the opposite side if additional transfers are to be made.

Identification of Transgenic Mice and Rats

Tail samples (1-2 cm) are removed from three week old animals. DNA is prepared and analyzed by Southern blot or PCR to detect transgenic founder (F_0) animals and their progeny (F_1 and F_2). In this way, animals that have become transgenic for the desired genes are identified. Because not every transgenic animal expresses the transgene, and not all of those that do will have the expression pattern anticipated by the experimenter, it is necessary to characterize each line of transgenic animals with regard to expression of the transgene in different tissues. Regarding this invention, the reporter gene is to be expressed in a particular tissue that will subsequently become neoplastic either by genetic predisposition, exposure to carcinogenic agents, infection with viruses, or other methods described in this application.

Production of Non-Rodent Transgenic Animals

Procedures for the production of non-rodent mammals and other animals have been discussed by others (46, 47, 54-62).

Identification of Other Transgenic Organisms

An organism is identified as a potential transgenic by taking a sample of the organism for DNA extraction and hybridization analysis with a probe complementary to the transgene of interest. Alternatively, DNA extracted from the organism can be subjected to PCR analysis using PCR primers complementary to the transgene of interest.

Developing the tumor

Various approaches for generating animals with an established preneoplastic state have been described above.

The establishment of the preneoplastic state in affected tissues is determined by standard histopathological methods (63).

Tumor development can be induced by a number of methods, also as described above. Tumor development, of course, need not be accelerated by active intervention, but rather may occur simply as the result of a secondary event occurring with the passage of time.

Tumor development is established by the presence of foci of neoplastic cells or proliferative lesions in histological analysis of tissue sections.

Methods for Reversion of Phenotype & Restoration of Reporter Gene Expression

A number of different approaches may be employed to test the model for sensitivity to the reversal of the transformed phenotype. For example:

1) It has been demonstrated that Ha-ras p21 expression can be modulated in vivo in cells in culture by exposure to antisense ras oligodeoxyribonucleotides (64). The administration of antisense oligonucleotides into animals (65) has been reported to inhibit oncogene expression (66).

2) Various temperature sensitive oncogenic proteins and viruses have been reported: SV40 T antigen (67); v-abl (68); p53 (69) & Ki-MSV (70). The establishment of tumors using

these oncogene forms permits the regulation of the activity of the expressed oncoprotein by temperature regulation in selected tissues in the intact animals (71).

3) In a number of cultured transformed cell lines , mouse embryonal carcinoma (72), murine erythroleukemia (MEL) (73) and colon carcinoma lines (74) treatment with differentiation promoting agents like Hexamethylene bisacetamide (HMBA) (75) resulted in the reversal of the transformed phenotype and the commitment to differentiation. In embryonal carcinoma cells a 30 hr treatment with 5 mM HMBA produced a restoration of α-Tropomyosin synthesis (72). In clinical trials HMBA was found to be effective in producing a partial remission of some cancers (76).

4) A variety of oncogene specific inhibitors have been reported in the literature. For example, Herbimycin A which specifically inhibit the tyrosine kinase activities of the oncogenes src, yes, fps, ros, abl and erbB has been reported to be effective in reversing the transformed phenotype produced by these oncogenes as well as some human colon tumors (77, 78, 79). Similarly, Lovastatin which interferes with the posttranslational modification of the Ras precursor protein inhibits ras activation in mammalian cells (80).

Expression of tumor suppressor genes can be altered as described above.

<u>Assembly of the final product</u>

Transgenic animals that have integrated the transformation sensitive promoter-reporter construct(s) (as described above) will be mated with animals that are predisposed to the development of tumors (as described above) to obtain test animals with a heritable preneoplastic state that have also incorporated a transformation sensitive reporter unit in its genome.

Protocols for Evaluating Putative Anticancer Agents

Assessments of efficacy and bioavailability of putative antineoplastic agents in test animals are performed following parenteral (Intraperitoneal, intramuscular, intravenous, intrathecal & subcutaneous injection), topical and oral routes of administration. Based on the pharmacokinetics profile, appropriate dosing schedules are instituted to maintain a threshold concentration (predetermined in in vitro assessment of effective concentrations). In vivo bioavailability and efficacy of the test agent(s) are evaluated by the restoration of normal reporter gene activity preferably in tissue sections, cellular homogenates or subcellular fractions of the tumor as an indicator of the reversal of the transformed state. Quantitation of the expression of the reporter gene(s) is preferably made by enzymatic methods (including the use of chromogens and chemiluminescence) which measure functional activity of the reporter gene product expressed, hybridization methods that determine the steady-state levels of the reporter gene transcript, and radioimmunoassay or immunohistochemical methods that detect the level of the reporter gene product(s).

Cell Culture Methods

Tissues from embryos or adults are dissected under sterile conditions such as in a laminar flow hood with sterile instruments. The tissue is chopped into small pieces with a sterile scalpel and subjected to disaggregation by trypsin digestion. After removal of large aggregates of tissue, the disaggregated cells are cultured in a rich medium including serum appropriate to the cell. Such procedures are well known to those skilled in the art of cell culture and can be found in standard text books on cell culture.

BEST MODE

In one example the test animal is obtained by generating separately a transgenic animal that has integrated into its

genome at least one copy of the transformation sensitive promoter-reporter gene construct and another transgenic animal that has sustained a genetic manipulation that renders some or all of the cells in the animal preneoplastic. The test animal that combines an inherited predisposition to develop tumor in the same cells or tissues that have also incorporated a functional transformation sensitive promoter-reporter transgene is obtained by crossing the two transgenic animals derived above.

For the construction of a transformation sensitive promoter-reporter construct, the regulatory region of the rat a-tropomyosin gene is fused to the bacterial B-galactosidase gene. The required rat a-tropomyosin promoter is obtained from the plasmid pTM1A. This plasmid contains a 6.5 kb fragment of the 5' region of the rat a-tropomyosin gene including exons 1, 2 and 3 (81, 82). A 4.2 kb BamH1-ApaI fragment containing the enhancer and promoter region and transcriptional start site was isolated by restriction endonuclease digestion. The ApaI end was then modified by conventional recombinant DNA techniques (41) using deoxyribonucleotide linkers to generate a SalI site. The B-galactoside gene was isolated as a 3.7 kb XhoI-HindIII fragment from the plasmid pCMV-B (Clontech). The a-tropomyosin-B-gal fusion construct is assembled by ligating the promoter and reporter gene fragments at the SalI/XhoI sites as shown in Figure 1 so that the reporter gene is operably linked to and whose expression is therefore placed under the control of the α-Tropomyosin regulatory sequence.

The fusion α-Tropomyosin-ß-gal construct was transfected (by Calcium Phosphate precipitation) into NIH3T3 and or Rat2 cells in culture. Clonal lines that have stably integrated the fusion reporter gene were analyzed for the expression of the B-galactosidase gene as evidence of the functionality of the α-Tropomyosin promoter in the construct. This is performed by Northern hybridization using a B-galactosidase specific probe and by histochemical assay

in the presence of the chromogen X-Gal (5-bromo-4-chloro-3-indoyl-B-D-galactoside) for the expression of the B-Gal mRNA and protein respectively. The sensitivity of the α-Tropomyosin regulatory region to cellular transformation will be assessed in the clonal cell lines by the subsequent introduction of an activated oncogene sequence for example the T24 Ras (83) either by conventional Calcium Phosphate transfection or retroviral infection. The alteration of expression of the B-Gal reporter in these Ras transformed cells will be documented by Northern blot analysis of message expression and X-Gal staining for functional reporter protein expression. The system will be further challenged by subjecting the cells to a reversion of the transformed phenotype by exposure to Ras antisense oligonucleotides at a concentration that has been previously found to inhibit H-Ras expression (84). The alteration of the reporter gene expression following reversal of the transformed phenotype will be verified by measuring B-Gal mRNA in Northern hybridization and B-Galactosidase activity.

The α-Tropomyosin-ß-Gal construct will then be introduced into animals for example by nuclear injection or ES cell technology. Transgenic animals that have integrated the promoter-reporter construct into their germ line are identified by Southern hybridization and PCR techniques. The animals are bred to obtain progeny that will be examined for expression of the reporter gene in the appropriate tissues (85, 86). Tissues or whole embryos will be fixed in 0.2% glutaraldehyde/1% formaldehyde. Alternatively, adult animals will be perfused with 4% paraformaldehyde as previously described (87). Expression of B-gal will be analyzed by incubation with the chromogenic substrate X-Gal which results in the deposition of blue stain. Expression of the B-gal protein can be analyzed by incubation of sectioned tissues with antibodies specific for the protein. The required antibodies can be obtained from Boehringer Mannhein.

In another animal, a heritable preneoplastic condition is

established by the mutational inactivation of p53 activity (88, 89). This is accomplished through the expression of an inactivated tumor suppressor gene product (90). In one example the p53 sequence is altered by oligonucleotide site directed mutagenesis (91) to obtain a mutant p53 that has sustained an inactivating mutation that substitutes an arginine for a histidine at position 175. The mutation will be verified by DNA sequence analysis (92). This mutation has been shown to result in an inactive p53 product with an increased efficiency for cooperation with the Ras oncogene in transforming primary rat cells in culture (93, 94). The expression of the mutant p53 gene or cDNA is placed under the transcriptional control of a strong ubiquitously active promoter like the Cytomegalovirus early promoter (CMV) (95, 96) or B-actin promoter (97). This is obtained by subcloning the 1.9kb Xbal fragment of the mutated human p53 cDNA (98) into a CMV vector (e.g. pCMVB) downstream from the 620bp CMV early promoter as shown in Figure 2. The p53 sequence can similarly be cloned downstream from a B-actin promoter.

The efficiency of this construct to direct the synthesis of the mutant p53 is initially assessed by transfecting the construct into NIH3T3 or Rat2 cells in culture. The stable expression of the mutant p53 in clonal cell lines is examined by both Northern blot and p53 specific immunoblot analyses. The ability of the mutant p53 product to interfere with the normal tumor suppressor function in these cells is assessed for by its cooperative effect with an activated oncogene e.g. H-ras to bring about an increased frequency of foci formation. This phenomenon is well documented (25).

A transgenic animal bearing the mutant p53 construct is obtained for example by nuclear injection or ES cell technology. Transgenic animals that have incorporated the fusion gene construct will be identified by Southern blot or PCR analyses as previously described. The expression of the mutant p53 transgene will be examined by Northern hybridization analysis of RNA from various tissues. The

identity of the mutant p53 message will be further verified by RNAse protection analysis (99). Other p53 mutants expressed in transgenic mice have been shown to result in the development of dysplasia in a number of tissues with a predisposed low frequency for the development of Lung, Bone and Lymphoid tumors (26). In another example, in which the p53 gene is inactivated by gene interruption (100) animals were also predisposed to the development of tumors. Transgenic animals that have integrated the mutant p53 construct in our experiments will be examined for the development of tissue dysplasia and tumor by conventional histopathological methods (101). The propensity of the cells in these animals to develop tumors at high frequency is examined by the introduction of an activated oncogene e.g. H-ras either through retroviral transduction or performing crosses with the Leder H-ras oncomouse.

The test animal that incorporates both the transformation sensitive α-Tropomyosin-B-Galactosidase reporter construct and the mutant p53 gene is obtained by mating the two types of transgenic animals above upon attainment of sexual maturity.

Tumor development in the above animal is accelerated by infection with a recombinant retrovirus carrying an activated oncogene like H-ras and examining for reporter (B-Gal) expression in tissue sections of sample tumors. In addition the alteration of reporter gene expression is followed upon treatment of the animal with known antineoplastic agents that act either directly on the oncogene product (102), interfere with the oncogene expression (e.g. Ras antisense oligonucleotides) or affect specific biochemical targets downstream from the oncogene signalling pathway (103).

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CLAIMS

1. A transgenic, nonhuman mammal having a transformation sensitive reporter unit at least a portion of which is introduced stably into the mammal, or an ancestor of the mammal.

2. A transgenic mammal as claimed in claim 1 wherein the reporter unit includes a regulatory region that is repressed upon neoplastic transformation of cell of the mammal.

3. A transgenic mammal as claimed in claim 2 wherein the regulatory region includes a transformation sensitive component that is present in the regulatory region of a gene selected from the group of genes consisting of: α-Actin, Myosin heavy chain, Myosin light chain, α-Tropomysin, Myo D1, Myogenin, Collagen alpha 2, H-2K (class 1MHC), Neu and Polyoma.

4. A transgenic mammal as claimed in claim 2 wherein the reporter unit includes an exogenous reporter structural gene.

5. A transgenic mammal as claimed in claim 4 wherein the reporter structural gene is selected from the group consisting of ß-galactosidase, chloramphenicol acetyltransferase, luciferase.

6. A transgenic mammal as claimed in claim 5 wherein the reporter unit is the regulatory region of α-Tropomyosin operably coupled to the structural gene encoding ß-galactosidase.

7. A transgenic mammal as claimed in claim 1 wherein the reporter unit includes a regulatory region that is activated upon neoplastic transformation of the mammal.

8. A transgenic mammal as claimed in claim 7 wherein the regulatory region includes a transformation sensitive component selected from the group consisting of regulatory regions of the following genes:

GLUTI and MDRI

9. A transgenic mammal as claimed in claim 8 wherein the reporter unit includes an exogenous reporter structural gene.

10. A transgenic mammal as claimed in claim 9 wherein the reporter structural gene is selected from the group consisting of ß-galactosidase, chloramphenicol acetyltransferase, luciferase.

11. A transgenic mammal as claimed in claims 1-10, wherein the mammal has a predisposition to develop neoplasia.

12. A transgenic mammal as claimed in claim 1 wherein the transformation sensitive reporter unit includes a regulatory region and a structural reporter gene that are operably coupled to one another and introduced as a coupled unit into the mammal, or ancestor of the mammal, at an embryonic stage.

13. A method for testing the ability of a drug to interfere with the onset or development of neoplasia comprising

administering to a mammal containing cells that have undergone cellular transformation a drug, and

determining whether the drug has affected the expression of a structural gene that is part of a transformation sensitive regulatory unit by assaying for the expressed product of the structural gene.

14. A method as claimed in claim 13 wherein the drug is administered to a transgenic nonhuman mammal that has a transformation sensitive reporter unit at least a portion of which is introduced stably into the mammal, or an ancestor of the mammal.

15. A method as claimed in claim 14 wherein the reporter unit includes a regulatory region that is repressed upon neoplastic transformation of the mammal.

16. A method as claimed in claim 15 wherein the regulatory region includes a transformation sensitive component that is present in the regulatory region of a gene selected from the group of genes consisting of: α-Actin, Myosin heavy chain, Myosin light chain, α-Tropomyosin, Myo D1, Myogenin, Muscle diff., Collagen alpha 2, H-2K (class 1MHC), Neu and Polyoma.

17. A method as claimed in claim 14 wherein the reporter unit includes a regulatory region that is activated upon neoplastic transformation of the mammal.

18. A method as claimed in claim 17 wherein the regulatory region includes a transformation sensitive component that is present in the regulatory region of a gene selected from the group of genes consisting of:

GLUTI and MDRI

19. A method as claimed in claims 15 and 17 wherein the regulatory unit includes an exogenous reporter structural gene.

20. A method as claimed in claim 19 wherein the structural gene is selected from the group consisting of: ß-galactosidase, chloramphenicol, acetyltransferase, luciferase.

21. A method for testing the ability of a drug to interfere with the development of a neoplasia comprising

administering a drug to a transgenic, nonhuman mammal having a transformation sensitive reporter unit, at least a portion of which is introduced stably into the mammal, or an ancestor of the mammal, at an embryonic stage, and

determining whether the drug has affected the expression of a structural gene that is part of the reporter unit by assaying for the expressed product of the structural gene.

22. A method as claimed in claim 21 wherein the reporter unit includes a regulatory region that is repressed upon neoplastic transformation of the mammal.

23. A method as claimed in claim 22 wherein the regulatory region includes a transformation sensitive component that is present in the regulatory region of a gene selected from the group of genes consisting of: α-Actin, Myosin HC, Myosin LC, α-Tropomyosin, Myo D1, Myogenin, Muscle diff., Collagen alpha 2, H-2K (class 1MHC), Neu and Polyoma.

24. A method as claimed in claim 21 wherein the reporter unit includes a regulatory region that is activated upon neoplastic transformation of the mammal.

25. A method as claimed in claim 24 wherein the regulatory region includes a transformation sensitive component that is present in the regulatory region of a gene selected from the group of genes consisting of:

GLUTI and MDRI

26. A method as claimed in claims 22 and 24 wherein the regulatory unit includes an exogenous reporter structural gene.

27. A method as claimed in claim 26 wherein the structural gene is selected from the group consisting of: ß-galactosidase, chloramphenicol, acetyltransferase, luciferase.

28. A method for making a transgenic nonhuman mammal comprising introducing into the mammal, or an ancestor of the

mammal, at an embryonic stage a recombinant transformation sensitive reporter unit.

29. A method of providing a cell culture comprising

providing a transgenic nonhuman mammal, all of whose germ cells and somatic cells contain a recombinant, transformation sensitive reporter unit introduced into the mammal, or an ancestor of the mammal, and

culturing one or more of the somatic cells.

30. A cell derived from a somatic cell obtained from a transgenic, nonhuman mammal, all of whose germ cells and somatic cells contain a recombinant, transformation sensitive reporter unit introduced into the mammal, or an ancestor of the mammal, which cell contains the recombinant transformation sensitive reporter unit.

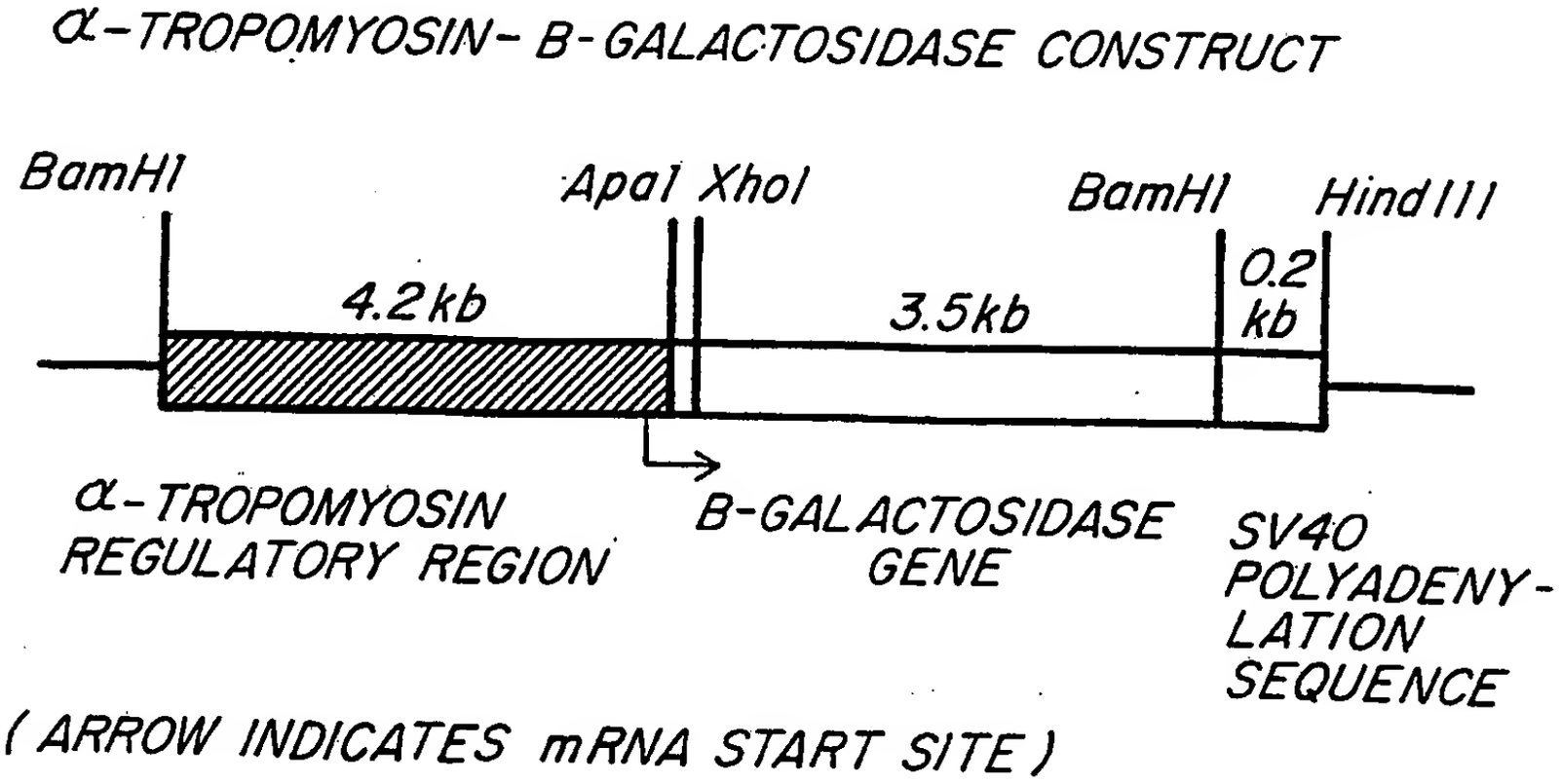
α-TROPOMYOSIN-B-GALACTOSIDASE CONSTRUCT
BamHI
ApaI XhoI
BamHI
HindIII
4.2kb
3.5kb
0.2 kb
α-TROPOMYOSIN REGULATORY REGION
B-GALACTOSIDASE GENE
SV40 POLYADENY-LATION SEQUENCE
(ARROW INDICATES mRNA START SITE)

FIG. 1

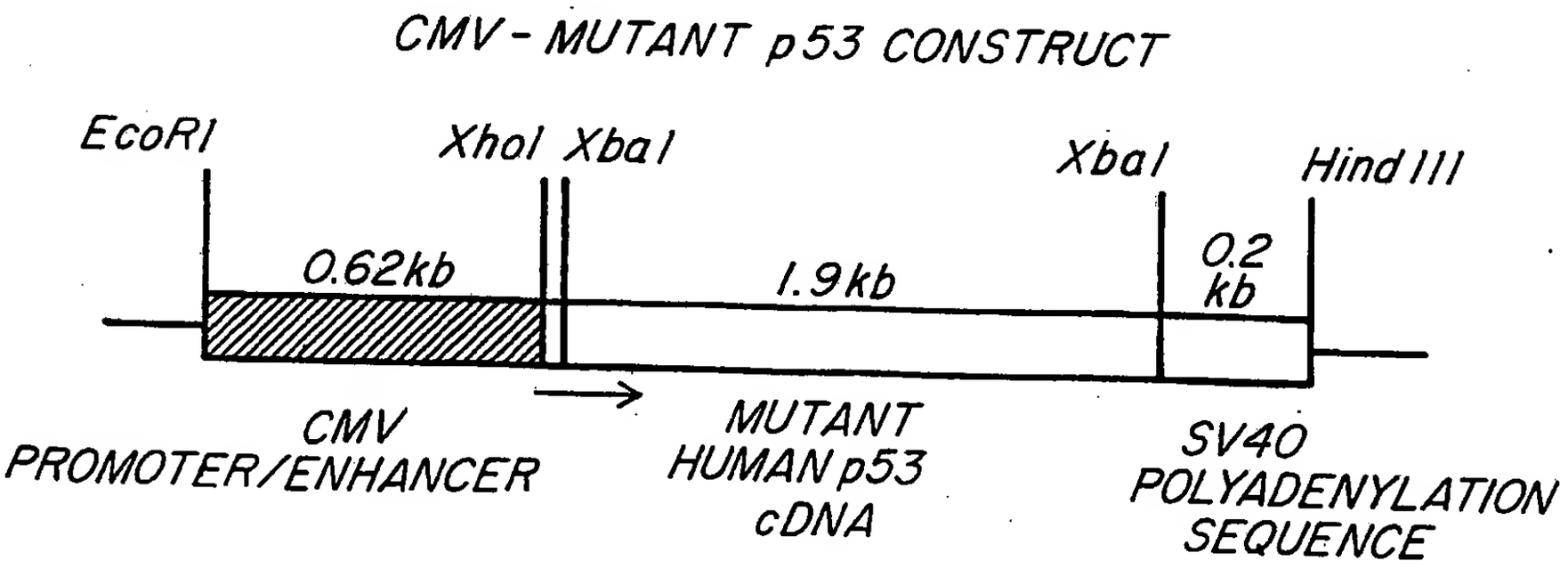
CMV-MUTANT p53 CONSTRUCT
EcoRI
XhoI XbaI
XbaI
HindIII
0.62kb
1.9kb
0.2 kb
CMV PROMOTER/ENHANCER
MUTANT HUMAN p53 cDNA
SV40 POLYADENYLATION SEQUENCE
(ARROW INDICATES DIRECTION OF TRANSCRIPTION)

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/04363

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/00; C12N15/85; A01K67/027; C12N5/10
G01N33/50; C12N15/12; C12N15/65

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A01K ; C07K ; C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO,A,9 012 093 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 18 October 1990	13
Y	see page 5, line 15 - line 19	1-7, 11-17, 19-24, 26-30
Y	PHARMACEUTICAL TECHNOLOGY vol. 15, June 1991, pages 26 - 32 KUMAR, C. C. 'Setting up reporter-gene based assay systems for screening antineoplastic drugs' cited in the application see the whole document	1-8, 12-17, 19-24, 26-30

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 AUGUST 1993	20 -08- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	CHAMBONNET F.J.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	WO,A,8 905 864 (THE TRUSTEES OF PRINCETON UNIVERSITY) 29 June 1989 see the whole document	1-30
Y	EP,A,0 370 813 (TRANSGENIC SCIENCES INC.) 30 May 1990 see the whole document	1-30
Y	WO,A,9 015 869 (EMBRYOGEN CORPORATION) 27 December 1990 see the whole document	1-30
Y	WO,A,9 004 632 (THE UNITED STATES OF AMERICA THE SECRETARY , DEPARTMENT OF COMMERCE) 3 May 1990 see the whole document	8,18,26
Y	WO,A,9 115 116 (AMRAD CORPORATION) 17 October 1991 see the whole document	11

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9304363 SA 73871

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 02/08/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		JP-T-	3502882	04-07-91
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		JP-A-	2145200	04-06-90
		JP-A-	4084900	18-03-92
WO-A-9015869	27-12-90	None		
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		EP-A-	0451157	16-10-91
WO-A-9115116	17-10-91	None		